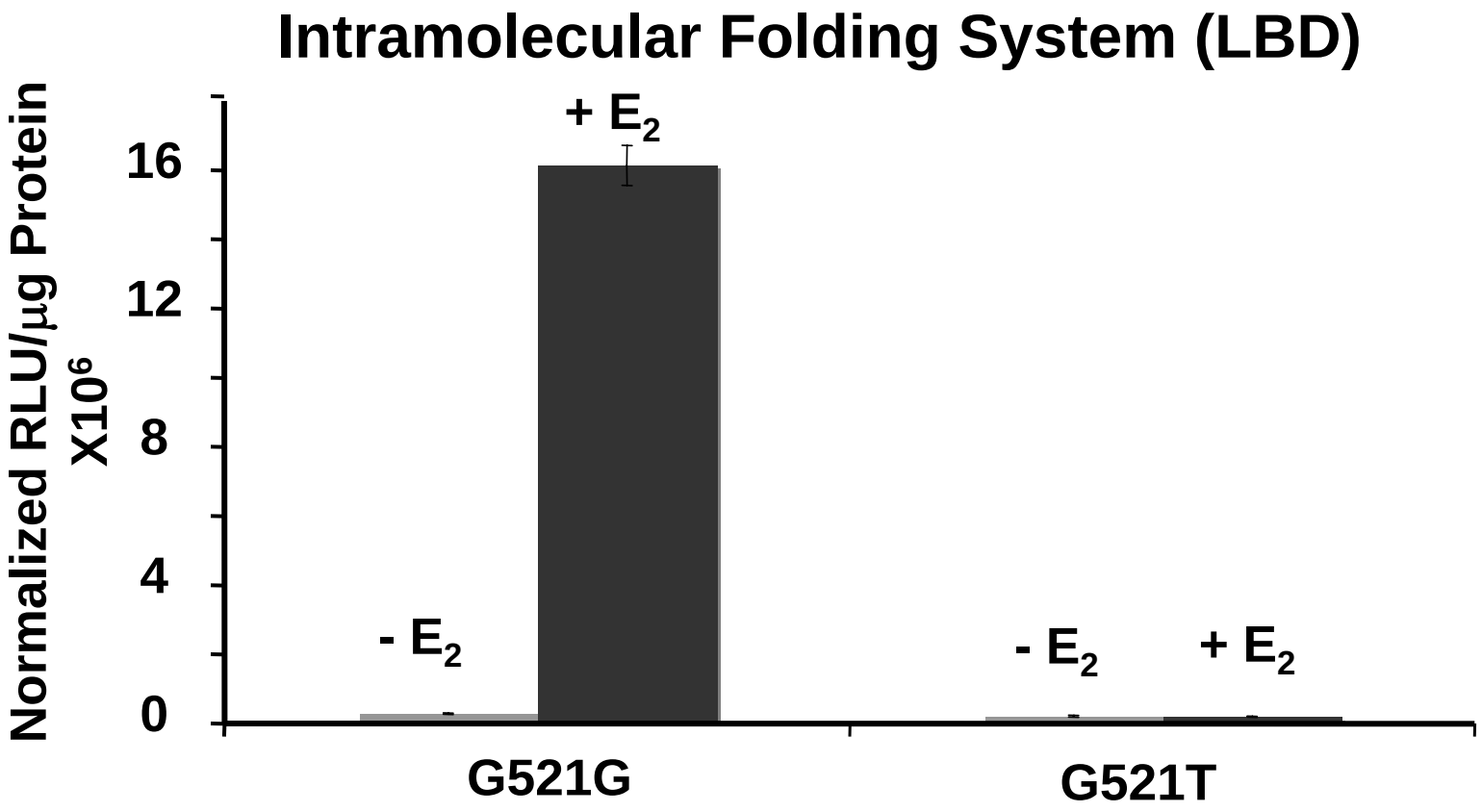

Supplemental Figure 1. Split firefly luciferase ER-intramolecular folding system with wild (G521G) and mutant (G521T) ER-LBD. The 293T cells transfected with the split firefly luciferase ER-intramolecular folding sensor constructed with wild and mutant ER-LBD (Fig. 1a, construct 3) were assayed for firefly luciferase activity with and without exposure to 1 μM of estradiol. Cells transfected with the sensor containing wild type ER-LBD show strong complemented luciferase signal when exposed to estradiol, whereas cells transfected with the sensor containing mutant ER-LBD show no signal both with and without exposure to estradiol.

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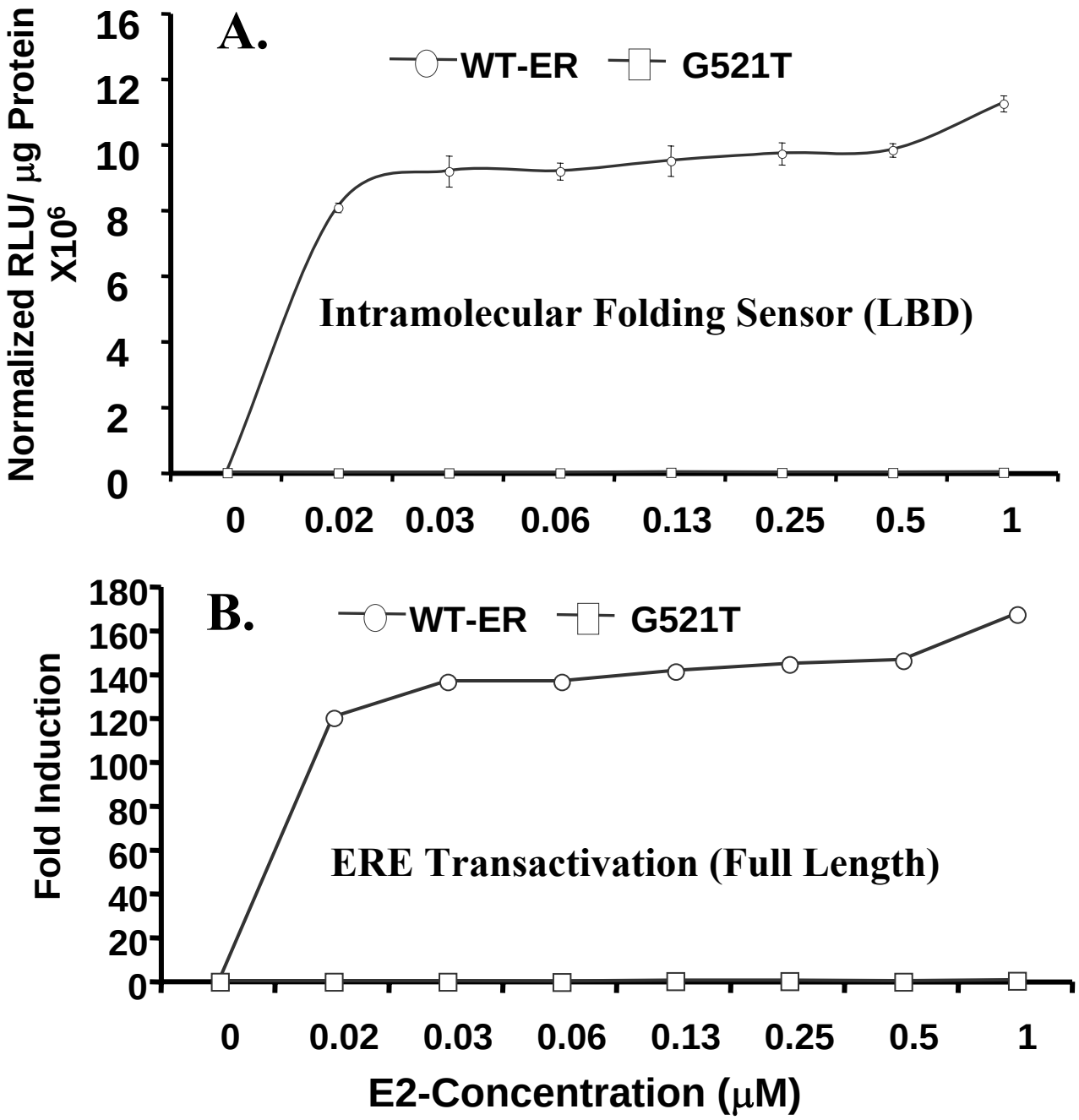
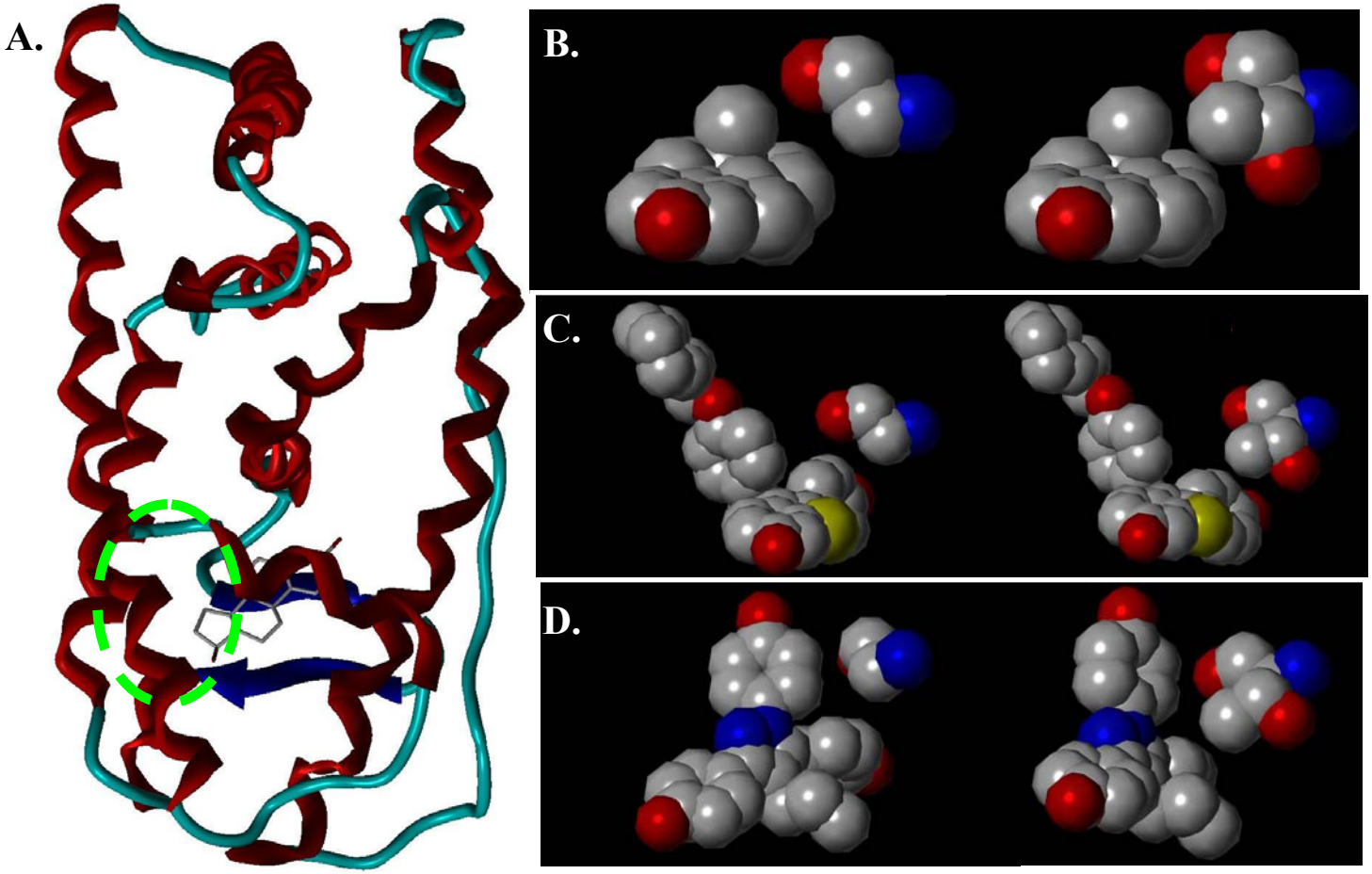
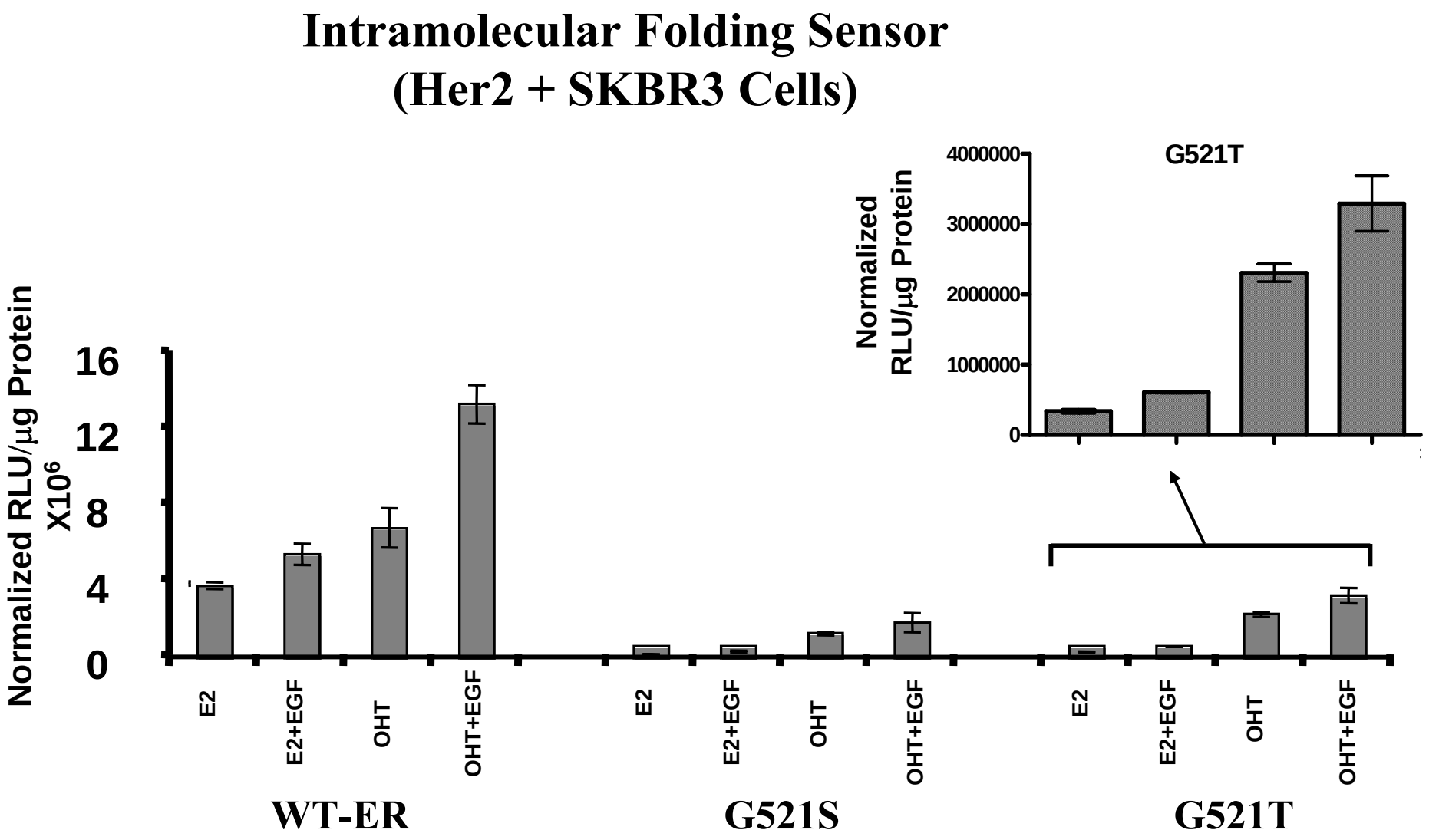


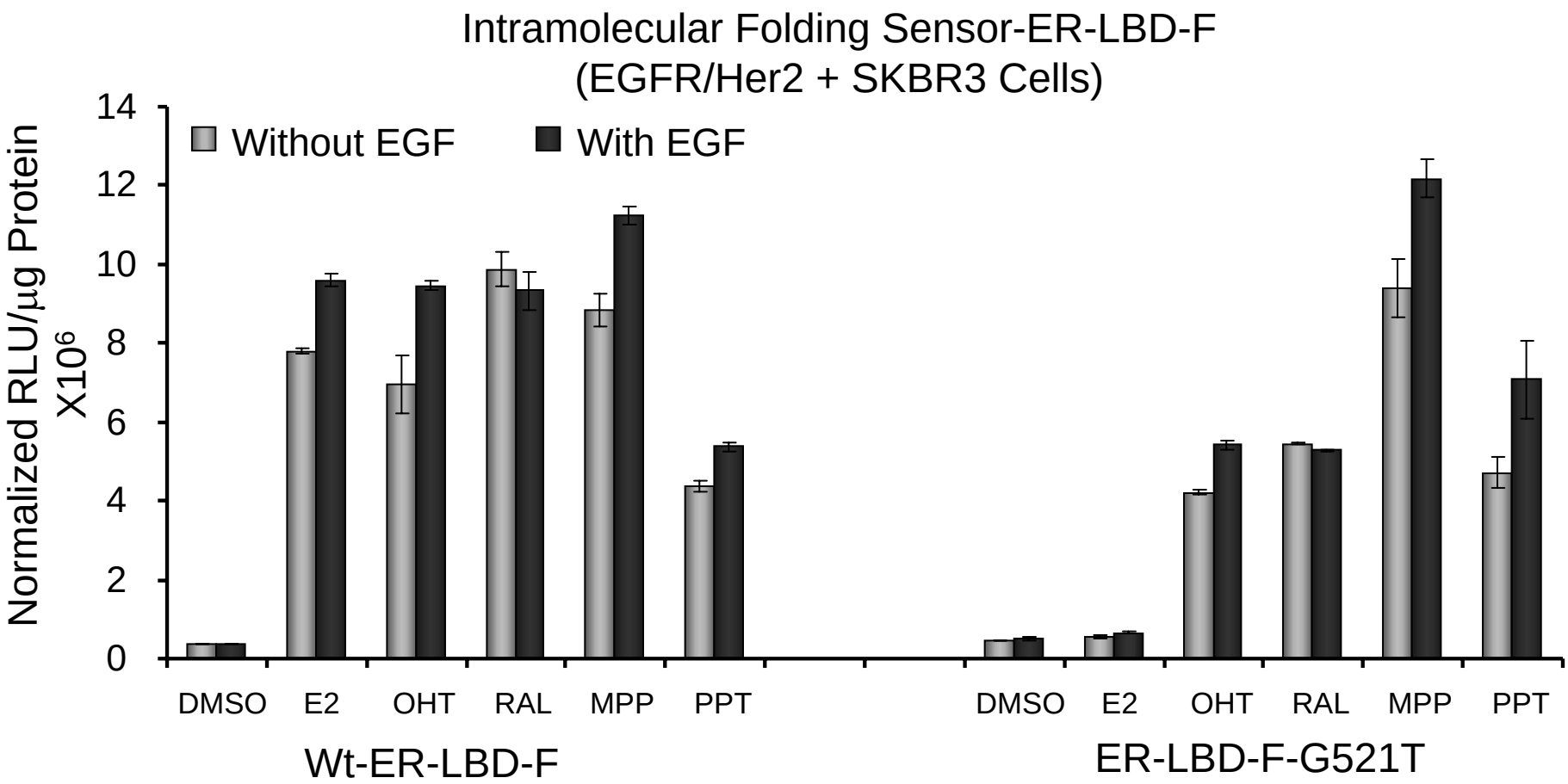
Supplemental Figure 2. Comparison of the response of the wild-type and (G521T) ER mutant intramolecular folding and the reporter gene activation assays to estradiol. *Panel A (Proteomic Assay):* The 293T cells were transfected with the split firefly luciferase ER-intramolecular folding sensor constructed with wild type and mutant ER-LBD (Fig. 1a, construct 3) and were assayed for firefly luciferase activity with and without exposure to different concentrations (0 to 1 μM) of estradiol. The cells transfected with the wild type ER sensor shows E_2 concentration-dependent increase in the complemented luciferase signal, whereas cells transfected with the mutant ER sensor shows no signal even at 1 μM E_2. *Panel B (Genomic Assay):* The 293T cells were co-transfected with either wild-type or mutant ER and an estrogen-responsive luciferase reporter gene (Fig. 1a, constructs 4 and 5) and were studied for reporter gene expression 24 hrs after exposure to different concentrations (0 to 1 μM) of estradiol. Only the cells co-transfected with wild-type ER show a ligand concentration-dependent activation of luciferase signal.



Supplemental Figure 3. Crystallographic images and models of ERα-LBD bound with estradiol, raloxifene and PPT. *Panel A:* The dashed oval region on the ribbon diagram indicates the position of G521 in helix 11 of the ERα LBD near the edge of the D-ring of estradiol. *Panel B:* A skeletal structure expanded view of the portion of and X-ray structure of the ERα ligand-binding pocket showing by space-filling the position of glycine residue 521 (*left)* and its steric proximity to two D-ring carbons (C-15 and 16) and the angular methyl group (C-18) of estradiol; when the larger threonine residue is modeled into this structure at 521 (*right*), a steric clash with these atoms of estradiol becomes apparent. *Panel C:* Details of the G521 residue (*left*) interaction with raloxifene in the crystal structure of ERα LBD; no steric strain is apparent in this structure nor in the model in which a threonine has been inserted at 521 (*right*). *Panel D:* Similar interactions of the ligand PPT with G521 (*left*) or T521 (*right*) in a model of this ligand in the ERα ligand binding pocket; again, no steric strain is noted in the T521 model. Figures were generated with Sybyl (version 7) (Tripos, St. Louis, MO) from the corresponding research collaboratory for structural bioinformatics protein data bank (RCSB-PDB) file name 1ERE for the structure with estradiol and 1ERR for the structure with raloxifene.



Supplemental Figure 4. Monitoring EGF-induced conformational changes in ER-LBD-F with renilla luciferase complementation within EGFR/Her2+ SKBR3 cells. Cells were transfected with wild-type or mutant ER conformational sensor (Fig. 1a, construct 1) and were exposed to 1 µM ligand ± 50ng/ml EGF. The increase in complemented luciferase activity (which is greater for OHT than E_2) is indicative of a conformational change in receptor upon EGF addition.



Supplemental Figure 5 Monitoring EGF-induced conformational changes in ER-LBD-F with renilla luciferase complementation in response to different ER-ligands within EGFR/Her2+ SKBR3 cells. Cells were transfected with wild-type or mutant ER conformational sensor (Fig. 1a, construct 1) and were exposed to 1 µM of different ligand separately ± 50ng/ml of EGF. The increase in complemented luciferase activity in different ligand treated cells after exposure to EGF is indicative of a conformational change in receptor upon EGF addition.

Supplemental Table 1.

		Biocharacter and Relative affinity	
S.No	Ligand	ER-α	ER-β
1	E1	Agonist,	Agonist,
2	Estradiol (E2)	Agonist, 100	Agonist, 100
3	E3	Agonist,	Agonist,
4	Tamoxifen	Mixed Agonist/Antagonist,	Mixed Agonist/Antagonist,
5	4-hydroxytamoxifen	Mixed Agonist/Antagonist, 140±24	Mixed Agonist/Antagonist, 62±8
6	Raloxifene	Mixed Agonist/Antagonist	Mixed Agonist/Antagonist,
7	DES	Agonist	Agonist,
8	DPN	No binding, 0.25±0.15	Agonist, 18±2
9	PPT	Agonist, 67±23	No binding, 0.14±0.05
10	MPP	Mixed Agonist/Antagonist, 9±4	No binding, 0.09±0.07
11	THC	Agonist, 28±7	Antagonist, 145±5
12	ICI 182, 780	Pure Antagonist, 32±14	Pure Antagonist, 25±0
13	Genistein	No binding, 0	No binding, 0
14	Cisplatinum	No binding, 0	No binding, 0

Supplemental Table 1. Biocharacter and relative affinity of different ligands used in this study to ERα and ERβ.